

Effect of olive mill waste (OMW) supplementation to Oyster mushrooms substrates on the cultivation parameters and fruiting bodies quality

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Abbreviations: DPPH: 2,2-diphenyl-1-picrylhydrazyl, MnP: manganese-dependent peroxidase, MiP: manganese-independent peroxidase, OMW: olive mill waste, WS: wheat straw

ABSTRACT

Seven Oyster mushroom strains were cultivated in wheat straw (WS) bags supplemented with 0 up to 90% olive mill waste (OMW), a solid residue obtained from a two-phases olive oil production system. All mushroom strains could grow but high OMW concentrations resulted in a significant yield, biological efficiency and productivity decrease, retarding of pinning and flushing and loss of fruiting bodies quality. However, most of the mushroom strains showed no significant differences on cultivation parameters and fruiting bodies quality (except for colour) between control (WS) substrates or substrates supplemented up to 50% OMW (w/w). Addition of 50% OMW to WS substrates turned mushrooms lighter and less reddish. This colour shift was not due to enzymatic oxidations or inhibition since laccase and peroxidase activities were not differing from control. Total phenolic content and antioxidant activity were also similar and no phenolic compounds from OMW were detected in the fruiting bodies. Some of the analysed *Pleurotus* strains were better suited to grow on OMW supplemented substrates than others.

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44 **1. Introduction**

45 The traditional olive oil production process (a three-phase continuous centrifugation
46 system) generates two types of wastes, an olive press-cake (OPC) and a dark liquid
47 effluent called olive oil wastewater or vegetation water (OMWW). Environmental
48 regulations encouraged producers to change their production systems because of the
49 large amount of highly polluting OMWW generated. These residues were directly
50 discharged in soils and receiving waters producing a strong negative impact on the
51 environment. Nowadays, modern olive oil factories (using a two-phase olive oil
52 production) discard a fibrous lignocellulosic paste with high moisture content called
53 olive mill waste (OMW), which includes a very high content of organic load and
54 harmful phenolic compounds too but in a lower volume than the traditional wastes.

55 To preserve the ecology of olive oil producers countries, degradation of these wastes,
56 particularly OMWW, have been intensively studied using thermal (combustion and
57 pyrolysis) and physico-chemical (e.g. precipitation/flocculation, ultrafiltration and
58 reverse osmosis, adsorption, chemical oxidation processes and ion exchange) processes,
59 agronomic applications (e.g. land spreading), animal-breeding methods (e.g. direct
60 utilisation as animal feed or following protein enrichment) and biodegradations in
61 bioreactors (by solid state fermentation using aerobic or anaerobic microorganisms)
62 (McNamara et al., 2008; Morillo et al., 2009). One of the most promising processes was
63 to decontaminate the OMWW using edible fungi or fungal enzymes. White-rot fungi
64 were able to secrete specific ligninolytic enzymes (polyphenol oxidases, peroxidases
65 etc.) causing significant phenolic removal (Fountoulakis et al., 2002; Aggelis et al.,
66 2003; Olivieri et al., 2006). Moreover, if edible mushrooms were utilized, a double
67 target could be achieved, on the one hand their enzymes were able to degrade OMWW

toxicity and on the other hand, they were able to produce sensory appreciated fruiting bodies (Zervakis et al., 1996). Apparently, mushroom species such as *Pleurotus* strains (Oyster mushrooms), *Agaricus bisporus* (common Button mushroom) or *Lentinula edodes* (Shiitake mushrooms) were able to colonize commonly used solid substrates supplemented with OMWW (up to certain concentration) and produce fruiting bodies with commercially acceptable yields (Kalmis and Sargin 2004; Kalmis et al., 2008; Altieri et al. 2009; Lakhtar et al., 2010). Despite the large number of studies describing the capacity of the *Pleurotus* varieties (*P. sajor-caju*, *P. cornucopiae*, *P. ostreatus*, *P. pulmonarius*, *P. eryngii* etc.) to grow and detoxify OMWW only a few studies describe the possibility of cultivating *Pleurotus* on the waste obtained from the modern olive oil production process (OMW) although at the present, the major olive oil producing companies from the Mediterranean countries are shifting their systems from the three- to the two-phases process. Most of the latter studies were focused on the decontamination / degradation of the phytotoxic phenolic compounds by the mushroom enzymes (Saavedra et al., 2006; Sampedro et al., 2007) or on the effect of these compounds on the bacterial flora endemic from the *Pleurotus* cultivation substrate (Soler-Rivas et al., 2006). However, the effect of the OMW supplementation on the *Pleurotus* growth and quality of produced fruiting bodies was not evaluated. On the other hand, the previous studies describing the OMW effect used only one or two *Pleurotus* species and results might largely differ depending on the selected strain since each individual variety has its own specific substrate requirements and capabilities.

If edible mushrooms can be cultivated on olive mill wastes, it is wise to take into consideration the fact that OMWs have toxic compounds harmful for human consumption (Aggelis et al., 2003; McNamara et al., 2008) and mushrooms grown on these substrates might absorb them and become toxic too. On the other hand, some of

the phenolic compounds present in the OMWs have been described as beneficial for human health (such as phenols with antioxidant activity) (Visioli et al., 1999; Mulinacci et al., 2001). Thus, absorption of these other compounds might have interesting influence on the fruiting bodies quality and nutritional value.

The most important quality parameters defined by the consumer in mushrooms are colour, texture and odour (Diamantopoulou and Philippoussis 2001; Caglarirmak, 2007). Good quality *Pleurotus* fruit bodies have to be off-white to brown, with tender but not soft texture, and have to possess the characteristic '*Pleurotus* odour' due to the presence of particular volatile compounds (Caglarirmak, 2007). Mushroom cap colour quality is also connected to the presence of oxidative enzymes. During mushrooms development, oxidative enzymes and its substrates are separated by membrane boundary layers within the cells. As a result of handling, natural ageing or infections, enzymes and substrates might intermix and enzymes become activated. Thus, enzymes such as peroxidases and laccases catalyze oxidative reactions yielding brown melanins and similar complex pigments contributing to quality loss.

The aim of the present work was to study the cultivation parameters and fruiting bodies quality of five *P. ostreatus* and two *P. pulmonarius* strains cultivated on wheat straw supplemented with several OMW concentrations (the waste obtained from two-phase olive oil production system).

2. Material and methods

2.1 Biological material

Mushroom strains used in this study were the commercial strains 2191, 2204, 2171 of *Pleurotus ostreatus* and *Pleurotus pulmonarius* 2204, obtained from the mother culture

collection of Mycelia co. (Gent, Belgium). *Pleurotus ostreatus*, strains K15 and *Pleurotus pulmonarius* P17, were supplied by the spawn company Fungisem S.A. (Autol, Spain). *Pleurotus ostreatus*, strain 1111 was kindly donated by INETI (Instituto Nacional de Engenharia Tecnologia e Inovação) (Lisboa, Portugal). Ordinary commercial Oyster mushrooms fruiting bodies were purchased at the local supermarket as standard mushrooms to compare the quality parameters evaluated. Olive mill waste (OMW) was obtained from a two-phase continuous olive oil mill plant extraction (Cooperativa de Olivicultores de Fatima, Fatima, Portugal) and stored at -25°C as it was acquired. Wheat straw used for mushroom cultivation was purchased at Hoge Born (Wageningen, The Netherlands). Wheat grains used to prepare spawn were supplied by Koopmans Meel BV (Leeuwarden, The Netherlands).

2.2 Culture media and spawn preparation

Subcultures of the mother mycelia from the commercial strains were grown on Petri plates containing 10 g L^{-1} of malt extract (Difco), 5 g L^{-1} of mycological peptone (Difco) and 15 g L^{-1} of agar (n° 3 Oxoid) (MMP medium). Mycelia incubation took place at 25°C . Spawn substrate was prepared by mixing rye grains with tap water in a ratio (2 : 1) (w/v) and sterilized in an autoclave 30 min. Sterilized spawn (20 g) was added to the top of colonised Petri plates (after 7 incubation days) to allow further mycelial colonisation of the grains. Plates were afterwards incubated at 25°C during 7 days. Afterwards, colonized grains were extracted from the plate and used to inoculate substrate bags.

2.3. Lab scale production of fruiting bodies

Specific substrate mixtures were prepared as follows: wheat straw was chopped (2 – 5 cm) and left overnight soaking up tap hot water. The excess of water was drained on a sieve during 20 min. Afterwards, the soaked straw was mixed and homogenized with 0% (control), 25%, 50%, 60%, 70%, 80% and 90% of OMW (expressed in dry weights of both straw and OMW). 100% OMW was also tested as substrate. Homogenized substrates (300 g) were placed in plastic bags (15 x 25 x 3 cm) and sterilized in autoclave during 30 minutes.

The sterilized substrates were inoculated with 10% (w/w) of fully colonized spawn from the selected strains and left incubated in dark at 25°C during 15 days. When substrates were fully colonized temperature was changed to 16 °C and 85% r.h., in the presence of light, in a day/night cycle and approx. 550 ppm CO₂ concentration to induce fructification. Two holes (approx. 2 cm diam.) were made in one side of each bag. After few days (depending on substrate and strain) pinheads were eye visible. Mushrooms obtained from the first and second flush were harvested and used to perform the below described experiments. Substrate bags were prepared in triplicate for each *Pleurotus* strain and for each type of substrate.

2.4. Evaluation of the cultivation parameters

Several cultivation parameters were evaluated during *Pleurotus* strains cultivation on control substrates (containing only wheat straw, WS) and on substrates including OMW supplementation. Moisture content and pH of the substrates were measured using standard methods (pH meter, drying procedure). The incubation time required before pinning (pinhead appearance) and for harvesting of the first and second flushes was recorded. Yield (expressed as grams of fresh mushrooms harvested at maturity per gram

of substrate, w/w), biological efficiency (BE%, defined as the percentage ratio of the fresh weight of harvested mushroom per gram of substrate (dw)) and productivity (BE% per day of mushroom production) were monitored and defined as described by Zervakis et al. (1996) and Curvetto et al. (2002). Other parameters taken into consideration were: average weight of individual mushroom strain (determined as the quotient between the total weight of individual strain harvested by their total number), and average dimensions (defined as the quotient between the fresh mushroom area of individual strain harvested by their total number) were evaluated according to Philippoussis et al. (2001) immediately after harvesting. Results were obtained from three replicates for each *Pleurotus* strain and for each type of tested substrate.

2.5. *Fruiting bodies texture*

Cap texture was monitored with a Texture Analyser (Stable Micro Systems, Godalming, UK). Measurements were performed immediately after harvesting and removing the stem. The cap was compressed in a distance of 1.5 cm from the edge, with a probe SMS P/0.25S at 1 mm s⁻¹ and 0.98 N force load into the fresh mushroom, according to the method of Diamantopoulou et al. (2001). Results were expressed as the force (N) needed to compress the cap and produce the first fracture in the mushrooms. Results were the average of ten measurements for each mushroom strain and each substrate.

2.6. *Fruiting bodies cap colour*

Cap colour was measured with a Minolta Chromometer CR-10 following the method of Soler-Rivas et al. (1997). Measurements were the average of six mushrooms per

strain and per type of substrate. The cap colour was evaluated immediately after harvested. The standardized $L^*a^*b^*$ scale system was used to define the colour.

2.7. Oxidative enzyme activities

Mushroom powders (166.7 mg mL^{-1}) were mixed with citric-phosphate buffer (0.1 M, pH 5) for laccase activity or succinic-lactic acid buffer (0.1 M, pH 4.5) for peroxidase activity, shaken on a vortex for 2 min, centrifuged (5 min, 14 000 rpm) and maintained on ice. Supernatants were used as enzyme source.

Laccase (Lac) activity was measured using ABTS (2,2'-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) as substrate and following the method of Gutierrez et al. (1994). Peroxidases activities (total peroxidases, manganese-dependent peroxidase and Mn-independent peroxidases) were measured following the method of Mata and Savoie (1998) using as substrate 3-Methyl-2-benzothiazolinone hydrazone hydrochloride (MBTH, Fluka) which interact with 3-dimethylaminobenzoic acid (DMAB, Aldrich) in the presence of the enzyme, and with/without H_2O_2 , manganese (Mn) or EDTA.

All enzymatic activities were monitored using a Perkin Elmer UV/vis Spectrometer Lambda 2S, by measuring absorbance of the oxidation products. The molar extinction coefficients of the oxidation products from ABTS and from MBTH/DMAB were respectively $\epsilon_{436} = 29\,300$ and $\epsilon_{590} = 32\,900 \text{ M}^{-1} \text{ cm}^{-1}$.

Enzymatic activities were defined as the amount of enzyme that transforms 1 mol of substrate second^{-1} (katal) per gram of dry weight of mushroom.

2.8. Phenolic compounds from mushrooms and OMW

Mushroom fruiting bodies cultivated and harvested as previously described where frozen and lyophilized (Soler-Rivas et al., 2006). Dried samples were ground on a mortar with liquid nitrogen and stored at -25°C for further use.

The total phenol concentration of mushroom powders (20 mg) was determined by the Folin–Ciocalteu method according to the procedure of Mau et al. (2002). Absorbance at 750 nm was measured in a Perkin-Elmer UV/Vis Spectrometer Lambda 2S. Gallic acid was used as standard for quantification.

The phenolic compounds characteristic of olive mill waste were evaluated on the prepared mushroom powders using the HPLC equipment (Waters) and methods developed and described in Soler-Rivas et al. (2006). Briefly, samples were injected onto a reversed phase HPLC column (Novapack C18 150 x 3.9 mm, Waters) and eluted with a flow rate of 1 ml min^{-1} and a mobile phase containing: (A) acetic acid/water (2.5%) and (B) acetonitrile following a gradient: from 0 to 10 min, 0 % B, from 10 to 40, 10% B, from 40 to 70, 40 % B, up to 72, 100 % B. Peaks were monitored using a diode array detector (Waters), identified on the basis of their retention times and spectra compared to standards and quantified at 280 nm.

2.9. DPPH scavenging capacity

Dried mushroom powders (25 mg), obtained as above described, were mixed with 1 mL methanol, shaken during 5 min in a Vortex and centrifuged for 5 min at 14 000 rpm. The antioxidant activity of the obtained supernatants (100 μl) was determined using a 1mM DPPH $^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) as radical solution according to Mau et al. (2002). Absorbance at 515 nm was measured after 30 minutes incubation.

3. Results

3.1. Effect of OMW addition on cultivation parameters

Control substrates containing only wheat straw showed a moisture content of 84.7% and a pH of 7.35 while fresh OMW showed 65.7% moisture and 4.9 pH. Thus, addition of OMW to the substrate induced a slight reduction of the water content (79.8, 72.9, 72, 72.4, 71.6 and 65% with respectively 25, 50, 60, 70, 80 and 90% supplementation) and a pH decrease (5.63, 5.34, 5.15, 5.20, 5.23 and 5.05 for these respective supplementation levels).

Several Oyster mushroom strains were cultivated on wheat straw mixed with several OMW concentrations. On average, addition of this residue up to 50 % (w/w) did not influence the incubation time required for pinheads appearance compared to control mushrooms cultivated on standardized substrate (Table 1). Pinheads from all mushroom strains were visible 17 – 21 days after spawn inoculation except for *Pleurotus ostreatus* 1111 which needed, on average, 30 days. Higher OMW supplementations (60 – 80%) provoked a generalized delay on pinning of approx. 4 - 7 days except for *P. pulmonarius* 2204 that was able to initiate fructification on 60% OMW at similar incubation time than control. None of the investigated strains was able to grow on 100% OMW and on 90% on average 30.6 days were required for primordia initiation.

On average, OMW additions up to 70% did not provoke a delay on the harvesting time for the first flush. All the fruiting bodies were harvested after approx. 6-7 days after pinheads appearance. The first flush of mushroom strains cultivated on substrates including OMW concentrations higher than 50% was harvested later, because they needed a longer interval to initiate fructification. Only OMW additions of 80 and 90%

OMW induced a flushing delay. The second flush was harvested earlier from those substrates containing higher OMW supplementation.

The mushroom yield obtained from the different substrates was also evaluated. Results showed similar profile than when their biological efficiency and their productivity were plotted. Mushroom yields on substrates including up to 50% OMW showed 0.13 g mushroom/g substrate meaning an 85% BE. Higher addition resulted in lower yields ranging from 0.09 up to 0.04 g/g (64 – 28% BE) with 60 up to 90% OMW supplementation. If the cultivation days were taken into consideration, the productivity of the cultivation process was, on average, slightly higher on substrates including 50% OMW than control substrates (Fig. 1). However, when individual mushroom strains were considered, productivity differences between 0, 25 and 50% OMW supplementation were not significant in four of the strains and in two of them the OMW addition was even detrimental (*P. ostreatus* 1111 and *P. pulmonarius* P17). Only for *P. ostreatus* K15 addition of 25 or 50% OMW provoked a remarkable increase on productivity. OMW additions at concentrations higher than 50% reduced the productivity of all the analyzed strains.

3.2. Effect of OMW addition on physical quality parameters

Under the described lab-scale cultivation conditions, *Pleurotus* mycelia grown on wheat straw were able to produce, on average, 20 fruiting bodies (in the two obtained flushes). Similar amount of mushrooms were harvested from substrate bags containing up to 50% OMW. Higher OMW supplementation resulted in lower amount of fruiting bodies (10 – 4) but of higher size. Mushrooms grown on 90% OMW showed a cap average size of 6.5 cm² while those grown on substrates with OMW concentrations

lower than 60% reached 3 cm². Thus, the total weight of harvested mushroom remained almost constant (6 – 8 g) since the amount of fruiting bodies was compensated by higher size. Only *P. ostreatus* K15 showed 1.3 and 3 folds higher fruiting bodies weight when it was grown respectively on 25 and 50% OMW addition compared to control substrate.

Fruiting bodies texture was also negatively influenced by OMW addition to the substrate provoking a softening of the mushrooms (Fig. 2). The firmness values recorded for all the analyzed *Pleurotus* strains were decreasing with increasing of OMW concentration in the substrate. However, for a few strains texture was not significantly affected by addition of 25 or 50% OMW.

The cap colour of the produced fruiting bodies (measured by the CIE *Lab* system) changed significantly and correlated to the OMW concentration added to the substrate (Fig. 3). This colour shifting was observed in all analyzed *Pleurotus* strains. The lightness (*L* parameter) changed, on average, from 60 up to 85 indicating that mushroom strains were getting lighter with increasing OMW concentrations in the substrate (Fig. 3a). The *a* parameter was also highly influenced since all mushroom strains shifted from red to green concomitantly with the increase of OMW addition (Fig. 3b). Changes on the *b* parameter with increasing OMW concentrations were strain dependent. Some of them such as *P. ostreatus* K-15 and 1111 turned bluer whereas other strains such as *P. pulmonarius* 2204 and *P. ostreatus* 2171 turned more yellow. Changes were larger when OMW was supplemented at concentrations higher than 50 or 60%. The rest of strains showed only slight changes on *b* parameter towards yellow (Figure 3c). Thus, when the total colour change was calculated as ΔE compared to control mushrooms (Figure 3d) a linearly correlated colour shifting was observed with the amount of OMW added to the substrate. Values were ranging from 5 to 20 (on

average) from 25 to 90% OMW. Total colour changes were particularly high in both *P. pulmonarius* strains.

3.3. Effect of OMW addition on chemical quality parameters

Pleurotus fruiting bodies showed both laccase and peroxidase activities. Laccase activity in all the studied strains ranged from approx. 1.5 to 3 nkat/g mushroom (dw) independently of the evaluated strain and OMW concentration in the substrate. Only the *P. pulmonarius* strains cultivated on substrates containing 80% OMW showed a significantly higher laccase activity than the others (Fig. 4a). The seven mushroom strains showed lower laccase than peroxidase activity being the latter influenced by the OMW concentration added to the substrate (Fig. 4b). No significant differences were observed between peroxidase activities in control mushrooms or mushrooms cultivated on substrates including 50% OMW. However, higher OMW additions resulted in higher peroxidase activities. Particularly, *P. ostreatus* strains showed an increase in their Mn-dependent peroxidase levels of approx. 2 to 4 folds the control or 50% OMW samples but no significant differences were found between *P. ostreatus* strains cultivated on 60, 70 or 80%. However, the values seemed to increase with OMW concentration in *P. pulmonarius* strains and in these strains, the increase was mainly due to increases on Mn-independent peroxidases (Table 2).

The total phenolic content and antioxidant activity of the seven mushroom strains did not significantly change with addition of 0, 50, 60, 70 or 80% OMW. Total phenol values were very similar for all the studied strains ranging from 0.13 to 0.22 mg/ml gallic acid equivalents. None of the phenolic compounds identified and detected in OMW (Soler-Rivas et al., 2006) was found on the fruiting bodies. Methanol extracts

obtained from *Pleurotus* fruiting bodies (independently of the substrate mixture used for cultivation) were able to scavenge 45 to 70% of the DPPH radical under the assay conditions, only *P. pulmonarius* P17 showed a DPPH scavenging capacity of approx. 80%.

4. Discussion

OMW addition to wheat straw substrates provoked a slight reduction of the water content and a pH decrease. Moisture content was still in the optimal range for cultivation (above 70%) common for this and other agro-residues based substrates (Hernandez et al., 2003). The use of an alkaline medium was usually suggested to reduce competing microorganisms and possible contaminating deuteromycetes (Stölzer and Grabbe, 1991). However, no CaCO₃ or similar compounds were added during the substrate preparation because few years later, Hernandez et al. (2003) demonstrated that fungal or bacterial contaminants could be avoided by keeping the relative humidity of the fruiting room not higher than 85%. Moreover, *Pleurotus* mushrooms were able to colonize other substrates with similar pHs ranges (7.5 to 5.6) (Yildiz et al., 2002).

When other *Pleurotus* varieties were cultivated on mixtures containing OMWW (Kalmis and Sargin, 2004) a significant delay on the cropping periods was observed with increasing OMWW concentrations. Similarly, OMW supplementation in concentrations higher than 50% is not encouraged since they induced retarding of pinning and flushing and reduction on their yield, biological efficiency and productivity. However, if the OMW was applied at 50% or lower concentration, their biological efficiency was in the range of other agro-wastes tested as substrates to cultivate Oyster mushrooms such as mixtures of switch grass or cottonseed hulls (Royse et al., 2004),

hazelnut, tilia, European aspen leaves, paper waste, sawdust etc. (Yildiz et al., 2002) or viticulture wastes, paddy straw or sesame straw (Kurt & Buyukalaca, 2010). Therefore, the *Pleurotus* strains selected in this study could use this lignocellulosic waste including up to 50% OMW as substrate to grow and to produce fruiting bodies. One particular strain, *Pleurotus ostreatus* K-15, seemed to grow better than in a standardized commercial substrate such as wheat straw. Thus, the following point to study was the quality of those fruiting bodies produced from OMW. Two particular characteristics were considered as a 'quality mushrooms': i) that they showed a physical appearance similar to those commercially available and ii) that they did not absorb or modify their level of phenolic compounds due to OMW addition.

According to the results, OMW additions up to 50% did not significantly influence the fruiting bodies number, size or texture if compared with control samples cultivated on wheat straw substrates including no OMW or commercial strains purchased at a local supermarket; only cap colour was affected. Oyster mushrooms cultivated on substrates including 50% OMW turned less red and greener and they became lighter. This discoloration might not be considered as quality depreciation since the *Pleurotus* strains commercially available at the supermarkets showed a wide range of colours from white to dark brown and they are all easily accepted by consumers. However, the colour change indicates that the chemical composition of these fruiting bodies has changed. Melanins and melanin-complexes are the responsible compounds for the mushroom cap colour. In *Pleurotus* mushrooms, these brown pigments are generated by the oxidative reactions catalyzed by enzymes such as peroxidases and laccases on phenols or phenols-like compounds. Thus, in this case, the colour shift might be due to certain compounds absorbed directly from the OMW able to inhibit the oxidative enzymes since turning whiter and less red might indicate lower melanin production. However, no inhibitors of

oxidative enzymes have been previously described on olive oil or derivative products. On the contrary, polyphenol oxidases from edible mushrooms were able to effectively degrade olive-related phenolic compounds such as hydroxytyrosol, tyrosol, methylcatechol, etc (Canfora et al. 2008; Espin et al. 2001). So if these were absorbed by the fruiting bodies, a darkening of the caps should have been expected.

The total phenolic content, antioxidant activity and laccase and peroxidase activities from mushrooms cultivated on substrates including 50% OMW were similar to those with no OMW addition suggesting that neither oxidative enzyme inhibition nor significant absorption of phenol-related-compounds occurred. Moreover, tyrosol, hydroxytyrosol 4-methylcatechol, caffeic acid and other more complex molecules described in OMW (Soler-Rivas et al., 2006) were not detected on any of the fruiting bodies analyzed (including those cultivated on substrates with very high OMW concentrations).

A discolouration of the cultivation substrate or media was also observed when *Pleurotus* mycelia were grown on OMW and OMWW-containing substrates (Soler-Rivas et al., 2006) or OMWW-containing liquid cultures (Kissi et al. 2001; Fountoulakis et al. 2002). The colour reduction was suggested to be due to the degradation of lignocellulosic fibres containing aromatic phenolic compounds by the mushroom oxidative enzymes secreted to the cultivation media. In those cases, colour change correlated with total phenol degradation and with the increase on oxidative enzymes activities, therefore the discolouration mechanism occurring inside the mushroom fruiting bodies should be different. Moreover, mushroom fruiting bodies do not have the metabolic pathways necessary to absorb such complex phenolic compounds or lignin-like molecules. They can only secrete the oxidative enzymes and degrade these extracellularly.

Thus, it could be concluded that Oyster mushrooms can be cultivated on wheat straw substrates supplemented up to 50% OMW with no significant changes in their cultivation parameters or quality of the produced fruiting bodies, as only a colour change was detected. The reason for this colour change remains unclear and needs further investigation although it can be suggested that apparently it is not a direct effect of the phenolic compounds from the OMW. Some specific *Pleurotus* strains such as i.e. *Pleurotus ostreatus* K-15 are better suited to grow on this substrate than others.

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Table 1: Cultivation time (days) required for pinhead formation (days after inoculation until appearance of visible primordia) and for the harvesting of first and second flushes of seven *Pleurotus* strains cultivated in several substrates including mixture with different OMW supplementations.

	Strain	OMW (%)						
		0	25	50	60	70	80	90
Pinheads	Average	21	21.0	21.1	25.6	27.3	27.6	30.6
	PO 2191	21	21	21	29	35	38	40
	PO 2204	17.5	17.5	17.5	25	22.5	24	25.7
	PO -K15	21	21	21	29	29	36.5	41
	PO 1111	31.5	30.7	29	29	29	35	39
	PO 2171	21	21	21	25	27	34	36
	PP 2204	17.5	17.5	17.5	17.5	22	25.5	32.5
	PP -P17	17.5	18.5	21	25	26.5	0	0
1 st Flush	Average	28.2	27.4	27.2	32.2	32.6	38.3	43.1
	PO 2191	28.5	26.5	25.8	36	35	39.8	45
	PO 2204	24.5	24.5	24.5	30	28.5	36.3	43.5
	PO -K15	27.5	27.5	28	34.5	34.5	48.5	50
	PO 1111	40	36.5	33.8	33.8	37.5	37	42.8
	PO 2171	26.5	26.5	26.5	33	34.2	36.8	41.5
	PP 2204	24.5	24.5	25.5	26.7	26.7	31.5	36
	PP -P17	26	26	26	31.5	32	0	0
2 nd Flush	Average	47.4	46.7	44.5	48.5	48.4	51.2	53.8
	PO 2191	50	45	40	51	52.5	0	0
	PO 2204	43	45	40	43.5	42.5	47	0
	PO -K15	50	47	43.5	47.5	50	57.5	0
	PO 1111	54	55	57.5	53.5	54	57.5	57.5
	PO 2171	45	45	43.5	50	50	51	0
	PP 2204	43	42.5	40	42.5	40	43	50
	PP -P17	47	47.5	47	51.5	50	0	0
Pin to 1 st Fl.	Average	7.2	6.4	6.0	6.6	5.3	10.7	12.5
1 st F to 2 nd F	Average	19.2	19.3	17.3	16.3	15.8	12.9	10.6

Table 2: Mn-dependent (MnP) and Mn-independent (MiP) peroxidase activities (nkat g⁻¹) of seven *Pleurotus* strains cultivated in several substrates including mixture with different OMW supplementations.

OMW	PO 2191		PO 2204		PO -K15		PO 1111		PO 2171		PP 2204		PP -P17	
	MnP	MiP	MnP	MiP	MnP	MiP	MnP	MiP	MnP	MiP	MnP	MiP	MnP	MiP
0%	1.63	0.87	0.57	3.28	0.63	3.07	0.15	3.35	1.12	1.18	1.04	0.86	1.17	1.83
50%	1.59	0.86	0.69	2.31	0.46	1.29	0.10	3.10	1.10	1.00	1.32	2.28	1.22	2.58
60%	4.39	0.61	2.74	3.36	4.24	2.36	2.15	2.95	4.02	3.48	1.01	3.19	1.56	3.34
70%	4.79	0.71	1.16	3.14	3.76	1.54	3.02	3.58	4.22	3.18	1.46	4.24	1.64	6.71
80%	3.81	0.59	2.12	3.78	2.88	3.62	2.73	3.57	2.82	2.88	1.02	4.78	1.37	7.93

534

535 **Fig. 1:** Productivity (biological efficiency per cultivation days) of seven *Pleurotus*
536 strains cultivated on wheat straw supplemented with different OMW concentrations.

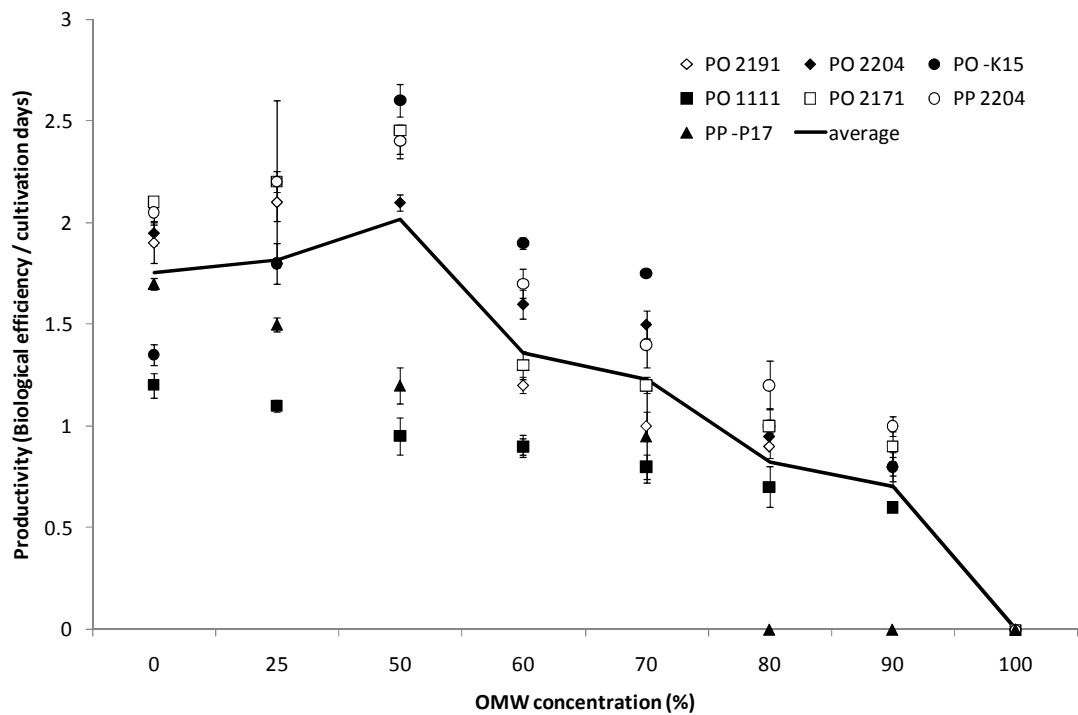
537 **Fig. 2:** Firmness values (N) measured in the seven *Pleurotus* strains grown on
538 substrates with increasing OMW concentrations and their average compared with the
539 average of commercial fruiting bodies.

540 **Fig. 3:** Cap colour measured in the seven *Pleurotus* strains grown on substrates with
541 increasing OMW concentrations and their average compared with the colour average of
542 commercial fruiting bodies. Values obtained for the a) *L*, b) *a* and c) *b* parameters and
543 d) total colour change defined as ΔE .

544 **Fig. 4:** a) Laccase and b) total peroxidase activities in several *Pleurotus* strains
545 cultivated in substrates with different OMW concentrations.

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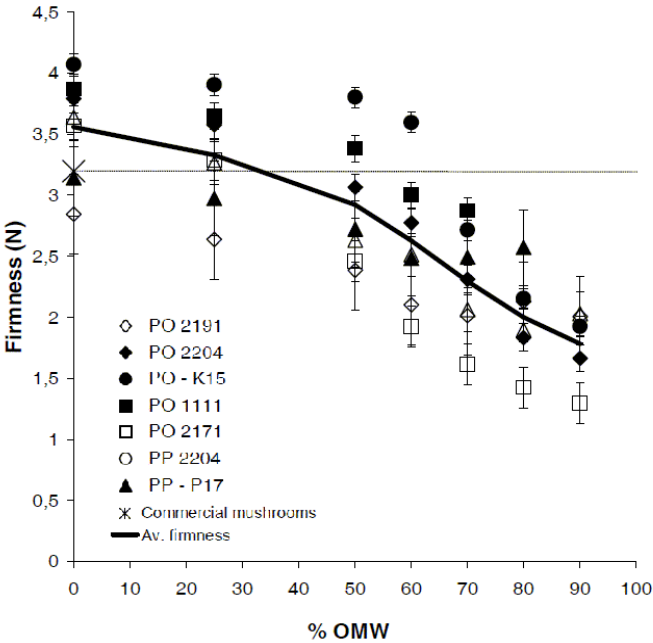
547 Figure 1:



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550 Figure 2:



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Figure 3:

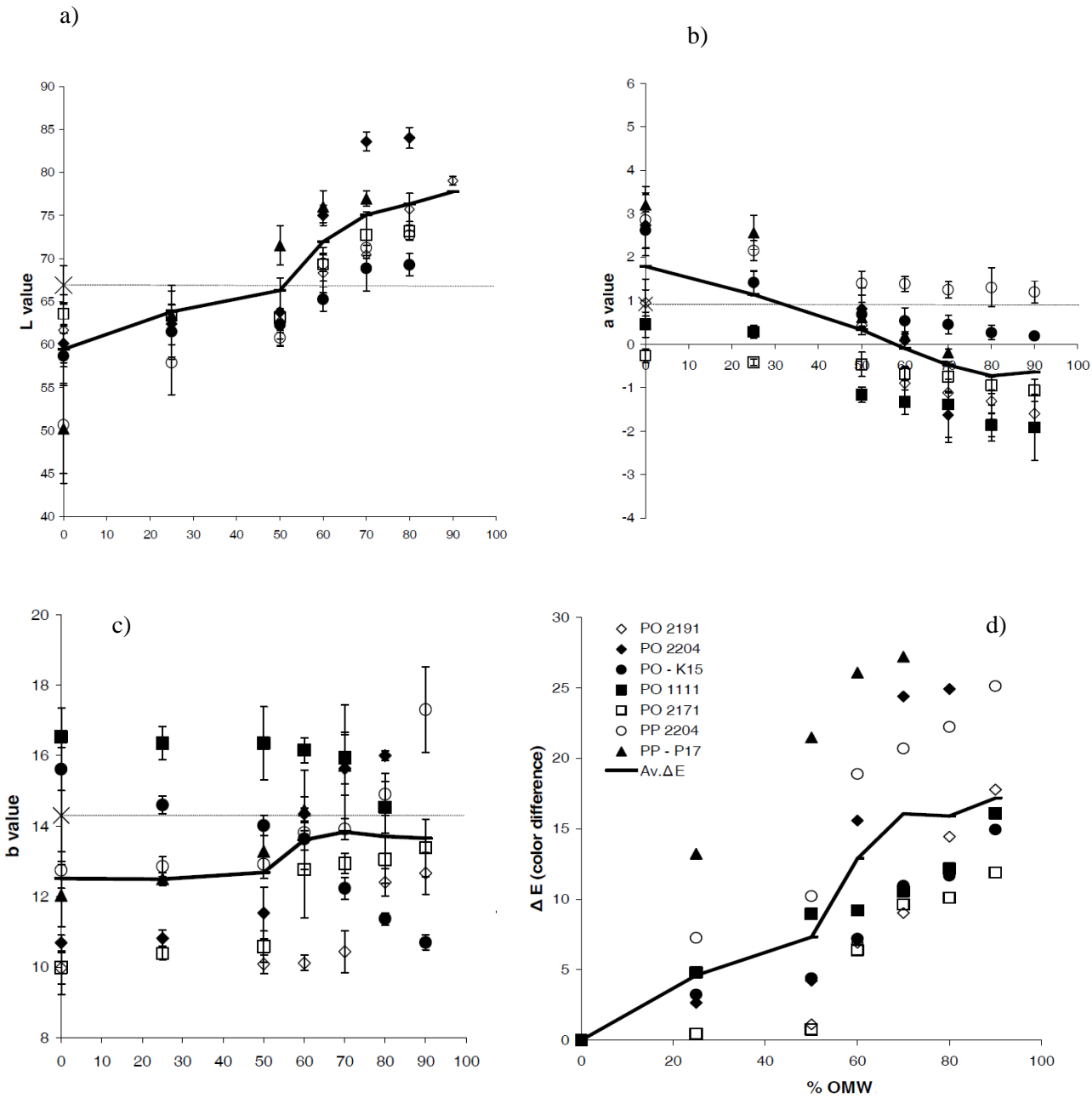
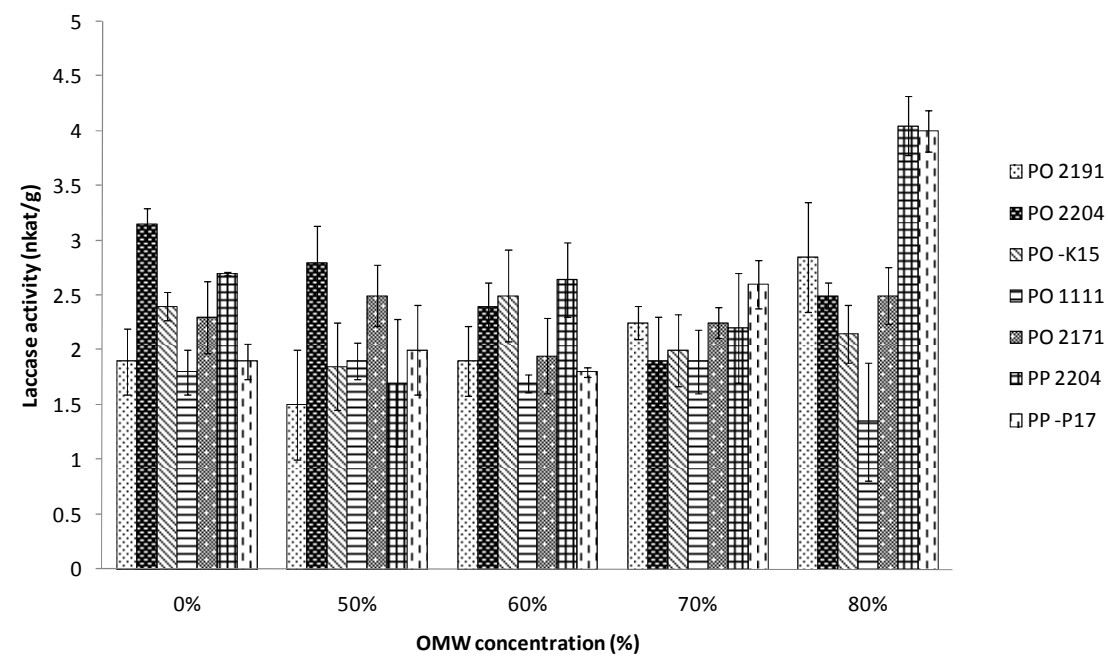


Figure 4:

a)



b)

